III. "A Contribution to the Study of the Yellow Colouring Matter of the Urine." By ARCHIBALD E. GARROD, M.A., M.D. Oxon., F.R.C.P. Communicated by Sir ALFRED B. GARROD, M.D., F.R.S. Received February 5, 1894.

The uncertainty which still surrounds the origin of a phenomenon so familiar as the yellow coloration of the urine bears eloquent testimony to the difficulties which beset the investigation, by ordinary chemical methods, of such substances as the urinary pigments, and to the importance of the part which the spectroscope has played in the acquisition of such knowledge of them as we possess.

Indeed, our acquaintance with the individual pigments is proportional to the distinctive character of their absorption spectra, rather than to the time which has elapsed since they first attracted attention; and in not a few modern works doubt is thrown upon the very existence of a distinct yellow pigment, having negative spectroscopic properties, but to which normal urine owes its characteristic tint, the chief part in the coloration of the excretion being assigned to urobilin.

In this connexion the spectro-photometric researches of Vierordt* are of much importance, for they appear to show conclusively that more than one pigment is present in normal urine. Vierordt found that with different specimens of the urine of a single healthy individual, examined at considerable intervals, the extinction coefficients for different parts of the spectrum exhibited relative as well as positive differences.

The variations of positive value are of course dependent upon the depth of colour of the specimen, but the relative variations can only be explained by the presence, in varying proportions, of two or more distinct pigments.

It must not, however, be forgotten that, as Vierordt himself points out, pigments which yield definite absorption bands may influence the extinction coefficients, even when present in such small quantities that their bands are not visible as such; and it can be shown that at least three colouring matters, apart from a yellow pigment, may be present in any given specimen of the urine of a healthy individual, which may, nevertheless, exhibit no obvious selective absorption.

Of these pigments, urobilin is certainly one, and when not seen on direct examination of the untreated normal urine, its band not infrequently appears on standing, or on the addition of a mineral acid.

Yet the quantity present is at best extremely minute, and wholly

* 'Die Quantitative Spectralanalyse.' Tübingen, 1876, p. 78.

inadequate to account for the coloration, and I am therefore convinced that the statement that urobilin is the chief colouring matter of normal urine is entirely incorrect. Indeed, as far as normal urine is concerned, urobilin can hardly be reckoned as one of its colouring matters at all, for even a very faintly tinted solution of this pigment yields a well-defined absorption band, far darker than is ever seen in normal urine. In some morbid urines, on the other hand, it affects the colour profoundly.

The second pigment referred to is hæmatoporphyrin, which, as I have elsewhere shown,* can usually be detected by appropriate means even in normal urine; but here, again, the amount present is so infinitesimal that it can have no appreciable effect upon the colour.

The occasional deposition of pink urate sediments, apart from any noticeable deviation from perfect health, shows that uroerythrin must also be reckoned among the pigments of normal urine; and if further confirmation is needed, it is obtained, as Riva† and Zoja have shown, by the examination of the extracts obtained by shaking specimens of urine with amylic alcohol.

Since, however, the above-mentioned pigments, with the possible exception of uroerythrin, can have no material influence upon the colour of normal urine, we are driven to the conclusion that there must exist in the urine another much more abundant colouring matter, of a yellow tint, which even in concentrated solution yields no absorption bands, or that the colour is due to the presence of more than one such substance.

There are not wanting records of investigations directed to the isolation of such a pigment, or mixture of pigments, and products have been obtained by several observers, which they have looked upon as the substance in question, but the various products have differed in their properties, and no one of them has met with general acceptance.

The literature of the subject will be found admirably epitomized in papers by Thudichum‡ and Schunck,§ published in 1864 and 1867 respectively, and to these epitomes there remains little to be added, seeing that during the twenty-seven years which have since elapsed, no fresh observer has, as far as I am aware, published any investigations upon the subject.

Referring my readers to these epitomes for records of the earlier work of Proust, Berzelius, Lehmann, Harley, and others, I only propose to allude here to the results obtained by Tichborne, Thudichum, and Schunck.

* 'Journal of Physiology,' 1892, vol. 13, p. 619.
† 'Gazzetta Medica di Torino,' 1892, vol. 43, p. 925.
‡ 'British Med. Journal,' 1864, vol. 2, p. 509.
§ 'Roy. Soc. Proc.,' 1867, vol. 16, p. 85.

C. Tichborne* (1862) threw down most of the colouring matter of a large quantity of concentrated urine upon a basic copper precipitate, and extracted the pigment from the precipitate by means of cold dilute sulphuric acid and alcohol.

In this way he obtained a solution which, on evaporation, left a brown residue, very hygroscopic and smelling of stale urine, solutions of which yielded, according to the degree of concentration, the various tints of normal urines.

The pigment was soluble to almost any extent in water, was insoluble in ether, sparingly soluble in absolute alcohol, and more readily in rectified spirit. It was precipitated from solution by basic lead acetate.

The results of elementary analysis led Tichborne to think that it was derived from hippuric acid by the subtraction of water, the percentage composition obtained being C, 67·80; H, 4·23; N, 8·56; O, 19·41.

It is extremely doubtful whether combustion analyses of such substances are calculated to materially advance our knowledge, in the absence of any of the ordinary guarantees of the purity of the substance analysed; and so simple a process as that employed by Tichborne could only be expected to yield a product of a moderate degree of purity.

Thudichum† (1864) obtained from normal urine by a variety of processes a substance to which he gave the name of urochrome, and his researches which have extended over a long period form the most elaborate contribution yet made to the subject.

In the second edition of his work on the urine,‡ in which his later researches are embodied, he gives four methods for the isolation of urochrome in which phosphomolybdic acid and the neutral and basic lead acetates are employed as precipitants, and sulphuric acid, sulphuretted hydrogen, &c., for the extraction of the pigment from the precipitates. Great pains were taken to obtain the pigment in the highest attainable degree of purity.

Thudichum describes urochrome as forming yellow crusts when its solutions are evaporated, as dissolving very readily in water, fairly readily in ether, and least easily in alcohol. It was precipitated from its solutions by lead acetate, silver nitrate, acetate and nitrate of mercury, &c.

On heating with mineral acids the aqueous solution became red, and resinous flakes were thrown down from which three definite substances could be obtained, which were minutely studied, and subjected to ultimate analysis. These substances were a red pigment,

* 'Chemical News,' 1862, vol. 5, p. 171.

† *Loc. cit.*

‡ 'Pathology of the Urine,' 2nd Edit., 1877, p. 217.

soluble in ether, with a port wine colour, and showing an absorption band to the more refrangible side of D (omicholic acid), a portion soluble in alcohol, showing a band extending from E to beyond F (uropittine), and a residue scarcely soluble in water or alcohol, but readily dissolved by alkalies (uromelanine).

Thudichum assigns to urochrome a faint absorption band at F, and was led to regard the pigment as a feeble alkaloid, on account of its precipitation by phosphomolybdic acid.

Schunck* (1867) employed the acetates of lead as precipitants, and extracted the colouring matter with cold sulphuric acid or sulphuretted hydrogen. He came to the conclusion that the urine owes its colour to two distinct yellow pigments, one soluble and the other insoluble in ether.

The pigment soluble in ether (urian) yielded, on heating with mineral acids, a brown resinous substance, readily soluble in alcohol (uroretine), whereas the pigment insoluble in ether (urianin) yielded a brown flocculent substance scarcely soluble in alcohol (uromelanine). He made numerous combustion analyses of these products, and his results differed widely from those of Tichborne, especially in the much smaller percentage of carbon found. For urian, the pigment soluble in ether, Schunck obtained the percentage composition C, 51·23; H, 5·38; N, 1·26; O, 42·13. Whereas urianin gave C, 46·44; H, 5·66; N, 3·16; O, 44·74.

In more recent years, Thudichum has on various occasions upheld the claims of urochrome to be regarded as a definite chemical entity,† in reply to the criticisms of Maly‡ and others.

Dr. Lewis Jones, who, some years ago, made some investigations on this subject, has favoured me with an account of his results, which were never published. He arrived at the conviction that the yellow colour of urine could not be due to urobilin, which, in solution, has a redder colour than urine. Moreover, the yellow pigment is insoluble in chloroform, in which urobilin dissolves freely. Urobilin, even in very dilute solution, has a very distinct absorption band at F, whereas normal urine shows no band at F unless viewed in deep layers, and then shows only a diffused obscuration about the region of the F line, quite unlike the sharp band of urobilin.

He found that an extract obtained by the lead acetate method, evaporated *in vacuo*, with proper precautions, yielded a yellowish crust, from which chloroform dissolved out any traces of urobilin. The remainder, when dissolved in water, reproduced the colour of the original urine when diluted to the same bulk. From normal urine the amount of urobilin obtained was very minute; more could be

* *Loc. cit.*

† 'Journal Chem. Soc.,' vol. 13, 1875, p. 392.

‡ 'Ann. der Chem. und Pharm.,' vol. 163, p. 90.

extracted from the highly-coloured urine of febrile patients, but in neither case does he consider that the quantity present suffices to materially affect the colour of the urine when diluted to the original bulk.

He adds: "I am disposed to regard the colour of urine as being due to the presence of a yellow body which, for the present, may be called urochrome, and, without positively denying the presence of traces of urobilin in normal urine, I consider that the amount which occurs in ordinary specimens is far too minute to affect the colour, whilst even in febrile urine the colour is only modified a little by the presence of urobilin."

The present writer was led to approach this difficult problem by the study of the coloration of uric acid sediments in urine, in which it became obvious that the yellow pigment played an important part. Attempts were therefore made to extract this pigment for purposes of further investigation, by a process which should differ from those hitherto employed in the following important respects:—

1. That, if possible, the recognised urinary pigments, and especially urobilin should be got rid of at the outset.
2. That the employment of powerful reagents, and especially of mineral acids, should be, as far as possible, avoided.
3. That the colouring matter should not be precipitated by lead acetate or other metallic compounds, and afterwards extracted from the precipitate.

After many attempts and repeated failures, a method was devised which, to a great extent, fulfils the above conditions, the essential parts of the process being as follows:—

1. Saturation of the urine with pure ammonium sulphate and filtration.
2. Extraction, from the filtrate, with ethylic alcohol, which separates out from the saturated liquid, and carries most of the colouring matter with it.
3. Evaporation, and solution of the residue in absolute alcohol.
4. Precipitation of the pigment from its alcoholic solution by excess of ether.

For purposes of more detailed description, it will be convenient to divide the process into the above four stages.

Stage I.—A pint or two of concentrated normal urine is saturated with pure ammonium sulphate, solution being aided by gentle warmth, and is then passed through a filter.

The filtrate is clear and has a pure golden colour, somewhat paler than that of the original urine.

The precipitate, which varies in tint from brown to pink, contains

any urobilin that may be present, as has been shown by G. Hoppe Seyler,* who makes saturation with ammonium sulphate the starting point of his process for the quantitative estimation of that substance.

Acidulated alcoholic extracts from the precipitate usually show a faint urobilin band, and sometimes still fainter bands of acid hæmatoporphyrin. Unusually pink precipitates will be turned green by alkalies, which shows that they contain uroerythrin.

If the precipitate be washed with water a yellow solution is obtained, which is found to contain some of the yellow pigment precipitated by the saturation.

Lastly, in addition to mucus, there may remain upon the filter paper a black residue, insoluble in water, alcohol, and dilute acids, but slightly soluble in soda, potash, or strong ammonia, which is an impurity derived from the ammonium sulphate.

A morbid urine, highly coloured with urobilin, yields a yellow filtrate, like that obtained with normal urine, which shows no urobilin band. I have reason to think that there is no such complete removal of hæmatoporphyrin, but any traces of this pigment which may exist in the filtrate are removed at a later stage.

Stage II.—To the saturated clear yellow filtrate absolute alcohol is next added, which throws down some of the ammonium sulphate, and after a small quantity has been added, quickly separates and collects upon the surface as a clear layer, carrying with it the bulk of the yellow pigment.

The alcohol is then separated off from the partially decolorised urine, from which a further supply of pigment can be obtained by a fresh addition of alcohol. By repeated extraction the pigment may be almost completely removed, but the result does not repay the expenditure of alcohol entailed. If rectified spirit be used instead of absolute alcohol, a considerably larger quantity is required to produce satisfactory separation.

The alcoholic extract thus obtained is next poured into a considerable bulk of distilled water, and the alcohol is again caused to separate out by once more saturating with ammonium sulphate, with the aid of gentle warmth. This washing process, which entails some loss of pigment, is of much importance, as by this means urea and other crystalline impurities are to a large extent got rid of; and its omission is apt to give rise to trouble at a later stage.

The golden orange-coloured extract thus obtained is inflammable, but will not mix with chloroform, as it still contains water and ammonium sulphate. It is therefore poured upon some fresh ammonium sulphate and gently warmed, when two layers will form, the lower of which is almost colourless, and represents much of the water pre-

* 'Virchow's Archiv,' vol. 124, 1891, p. 30.

viously contained in the extract, the bulk of the dissolved ammonium sulphate being separated with it.

Stage III.—The lower layer having been removed, the alcoholic extract is now evaporated to dryness over a water bath, a few drops of ammonia being added from time to time so as to maintain an alkaline reaction.

This precaution is rendered necessary by the presence in the extract of a considerable quantity of indoxyl sulphate, which is otherwise apt to be decomposed during the evaporation, with the formation of indigo pigments.

Such decomposition cannot take place if the liquid be kept alkaline, for, as Baumann showed, the indoxyl sulphates may be boiled with caustic alkalies without undergoing change ; and, far from producing any alteration in the yellow pigment, the ammonia tends to preserve it from changes to which it is otherwise liable.

A brown residue remains after the evaporation is complete, which has a treacly consistence, but solidifies on cooling. This residue, which emits a powerful urinous odour, and contains some ammonium sulphate, is washed once or twice with acetic ether, which removes the bulk of the indoxyl sulphate, and comparatively little of the yellow pigment. It is then transferred to a stoppered bottle and allowed to soak for some hours in absolute alcohol. On filtering a beautiful orange-coloured alcoholic solution is obtained, but some of the pigment escapes solution and may be in part removed by a second soaking in fresh alcohol.

Water dissolves the undissolved residue readily and completely, and if the aqueous solution so obtained is treated like the original urine by saturation with ammonium sulphate and extraction with alcohol, a further supply of absolute alcoholic solution may be obtained from it, which has the advantage of being free from indoxyl sulphate.

It is probable that some of the pigment has undergone a slight change which renders it very sparingly soluble in alcohol.

Stage IV.—The alcoholic solution, which, when treated in the cold with hydrochloric acid and a trace of chloride of lime, still yields an indigo reaction, is next concentrated, if necessary, until it has a rich orange colour. It is then poured into rather more than its own bulk of ether, whereupon much of the pigment is precipitated in an amorphous state, and may be collected upon a filter which has been first moistened with pure ether to ensure rapid filtration.

The presence of a very little water prevents the precipitation, a few drops of a very concentrated aqueous solution separating out and passing through the filter.

If the various stages of the process have been carefully followed, and especially if the second separation of the alcoholic extract from

distilled water has been carried out, the filter paper will merely be coated with an amorphous brown film, and there will be no appreciable deposit of crystalline impurities. The filtrate of ether and alcohol will have a yellow colour, as it is able to hold a considerable quantity of pigment in solution.

The filter paper, to which the pigment clings tenaciously, is allowed to dry and is then soaked for a time in chloroform which remains untinted, and afterwards in absolute alcohol. The alcohol becomes coloured by the pigment, but does not dissolve it nearly so readily as before.

The paper is then allowed to soak for an hour or so in distilled water, by which means a clear orange-coloured or yellow aqueous solution is obtained, which should yield no indigo reaction, and which contains the pigment in a condition approaching to purity, although it is certainly not entirely pure. I believe, however, that no pigment other than the yellow one is present, and the absence of those which show absorption bands can be definitely established.

When it is treated with sodium hypobromite, a little nitrogen is evolved, the freedom from urea being in proportion to the amount of washing or soaking in alcohol that the brown precipitate has received. If only slightly washed, 1 c.c. of a concentrated solution tested by means of Southall's apparatus, may give off nitrogen equivalent to as much as 0·003 gram of urea, but if the washing has been more thorough, the amount evolved is too small to be measured.

Seeing how soluble urea is in alcohol and in water, whilst it is almost insoluble in ether, it is only to be expected that this substance should constitute the chief impurity in the specimens.

When the residue obtained by evaporation of the aqueous solution is burnt on a platinum dish, it yields a very bulky mass of carbon, and ultimately a trace of colourless ash, varying in quantity, which is readily soluble in water, contains no appreciable amount of carbonate, and apparently consists of sodium phosphate.*

* *May 9th*, 1894.—It has been suggested that the yellow pigment may contain some, at least, of the iron which is present in urine, but although I cannot state that the product obtained by the above process is *absolutely* free from iron, the amount of that element contained in it is, at most, exceedingly minute. After the combustion of such small quantities as 1 or 2 cgrm. of the dry pigment, the sulphocyanide test gave negative results, provided that iron-free reagents, and filter-papers which had been extracted with hydrochloric acid, were employed in the process; but with as much as 6 cgrm. a just perceptible tint was obtained, in no way comparable with that yielded by a fiftieth part of that weight of hæmoglobin. I am, therefore, inclined to look upon this minute trace of iron as an accidental impurity, probably derived from the urine, rather than as a constituent of the yellow pigment.

When heated with potassium hydrate the yellow pigment was found to give off ammonia freely.

The final product represents but a small part of the yellow pigment present in the original specimen of urine, conspicuous loss being entailed in the washing of the alcoholic extract, the washing with acetic ether, and especially in the precipitation with ether.

I have not made any ultimate analysis of the product, since such an analysis would have little value without further guarantees of the purity of the product, and such guarantees could hardly be obtained in the case of a colloid substance such as this pigment is. A long series of combustion analyses, if they yielded uniform results, would doubtless go far towards establishing its percentage composition, but in order to obtain the material required for such a series, very large amounts of urine would have to be dealt with, and a correspondingly large consumption of the materials employed in the extraction of the pigment would be involved.

Properties of the Solid Pigment.

In the solid state the product obtained by the above process was completely amorphous and brown in colour. It was so hygroscopic that it could not be completely dried in air, but in the exsiccator, over sulphuric acid, it lost its viscosity, and became quite hard.

It dissolved in water with the greatest facility, readily in rectified spirit, and much less readily in absolute alcohol. Acetic ether, amylic alcohol, and acetone dissolved the pigment sparingly.

The solubility of the product in alcohol appeared to undergo a progressive diminution, through the successive stages of extraction, and after each evaporation of an alcoholic solution some of the pigment was apt to escape re-solution in alcohol.

In pure ether, chloroform, and benzene it was quite insoluble, but mixtures of ether or chloroform with alcohol dissolved it to some extent. In its purest state the pigment was practically odourless when cold, but on the water-bath it softened and emitted a slight urinous odour.

Properties of Solutions of the Pigment.

The solutions of the pigment in alcohol or in water reproduced on dilution the various shades of yellow and orange colour of normal urines. On concentration they passed through various shades of orange to a rich, warm brown.

Blue litmus paper dipped into the solutions was slightly reddened, and red litmus took a faint blue tint.

When the solutions were placed before the spectroscope they showed no absorption bands, even on the addition of an acid. The blue end of the spectrum was absorbed, and the absorption faded away so gradually towards the yellow, that even with concentrated solu-

tions it was not possible to assign to it even an approximate limit. There was no increase of the absorption in the position of the urobilin band.

Treatment with zinc chloride and ammonia did not produce any fluorescence.

The alcoholic solutions always showed the same rich yellow or orange tint, and could be kept for a long time without undergoing any appreciable change; but aqueous solutions kept in stoppered bottles tended to assume a brown tint on standing, even when dilute, and this change was precipitated by evaporation or warmth. In this respect my product behaved just like urochrome.

The tendency of the aqueous solutions to undergo this change could be restrained by the addition of a little ammonia.

Alkalies did not appreciably alter the tint of dilute solutions, but more concentrated ones were slightly browner when alkaline than in the neutral condition.

Small additions of mineral acid produced no immediate change, but larger quantities quickly changed the colour to a reddish-brown.

Solutions of the pigment were decolorised by nascent hydrogen produced by the action of hydrochloric acid upon zinc. This is only to be expected, seeing that it is a known fact that the urine is itself decolorised by similar treatment.* The destroyed colour was not restored by hydrogen peroxide.

Action of Mineral Acids upon the Pigment.

Solutions of the yellow pigment when warmed with nitric acid remained clear, but took a distinctly brighter yellow tint. On the addition of ammonia to alkalinity the yellow colour changed to a rich orange, the changes of tint being exactly similar to those which constitute the xanthoproteic reaction. This reaction seemed to be due to a change in the pigment as a whole, and not to any traces of impurity present.

Heated over the water bath with the addition of sulphuric or hydrochloric acid, the changes observed were uniform with all specimens of the indigo-free pigment which were subjected to this treatment, and were the same whichever of the two acids was employed.

The colour of the liquid quickly changed to reddish-brown, and on evaporation to dryness a nearly black residue was left. This residue, when treated with water, yields an orange-coloured solution, resembling the original liquid in colour, but darker in tint. This aqueous extract left on evaporation a brown residue, which was scarcely soluble in alcohol, but which communicated a yellow colour to chloroform.

* Salkowski und Leube, 'Die Lehre vom Harn,' 1882, p. 14.

From the remainder of the original residue alcohol extracted more pigment, and hot alcohol more still, the liquid assuming a sepia tint, and showing no absorption bands. The hot alcoholic solution deposited, on cooling, a dark, pulverulent sediment, which, examined microscopically, was found to be amorphous. A black residue still remained which was insoluble in water, alcohol, and dilute acids, was scarcely soluble in amylic alcohol, but was readily dissolved by strong ammonia (the uromelanine of Thudichum). The alkaline solution gave no absorption bands.

After extraction with water the original residue communicated a yellow colour to ether, but no substance resembling the omicholic acid of Thudichum (which is readily soluble in ether with a fine red colour) was obtained.

Precipitants of the Yellow Pigment.

In its behaviour towards metallic salts the pigment obtained by my process exhibited the closest resemblance to the urochrome of Thudichum.

The solutions were almost decolorised by the acetates of lead, by nitrate of silver, and by phosphotungstic and phosphomolybdic acids, which all threw down precipitates containing the bulk of the pigment.

Mercuric acetate decolorised the solutions completely, a yellow precipitate being formed, from which the colouring matter could be readily extracted with alcohol acidulated with hydrochloric acid, but apparently not without some change, evidenced by its reddish-brown colour.

Mercurous acetate had not the power of throwing down the pigment from its solutions.

Behaviour of the Pigment towards Uric Acid.

If to a solution of colourless urate, obtained from snake's excrement, some of the yellow pigment was added, and if the conditions of the experiment were so adjusted that crystals of uric acid are slowly deposited from the solution, these crystals resembled those which compose the yellow or brown variety of uric acid sand, and had, moreover, the ordinary urinary forms, the familiar whetstone shape preponderating. I have, indeed, specimens of crystals so obtained which are quite indistinguishable from those of the natural urinary sediments.

This experiment is difficult to carry out satisfactorily, chiefly owing to the instability of the isolated pigment. If the crystals are too quickly deposited they have the whetstone form, but are only

faintly tinted. If acid is added they have a brown colour like that of crystals thrown down on the addition of acid to urine.

The converse experiment to this was performed some years ago by Ord,* who showed that, on repeatedly redissolving and reprecipitating urinary uric acid, the crystals lost their colour, and, at the same time, tended to assume the tabular forms of those of pure uric acid.

The above result lends strong support to the view that the pigment is isolated by the alcohol process in the form in which it actually exists in the fresh urine, and confirms the statement that it plays an important part in determining the forms which the crystals assume.

Another fact which is demonstrated by this experiment is that the yellow pigment is one of those which colours the urinary crystals, although it does not stand alone in this respect. I do not, however, propose to enter further into this subject here, as I hope to deal with it at length in a separate paper, but I may mention that crystals of uric acid which are deposited from a solution of urobilin are colourless and exhibit no modification of form, resembling, in every respect, those thrown down from pure aqueous solutions of urates.

Summary and Conclusions.

There cannot, I think, be any doubt that the substance isolated from the normal urine by the process here described is that to which its colour is almost entirely, if not entirely, due, and, since solutions of this substance do not fluoresce with zinc chloride and ammonia, show no absorption bands, and cannot be got to show a urobilin band by any process to which it was subjected, it follows that urobilin is not the chief colouring matter of normal urine. Moreover, there is every reason to believe that the product obtained has not undergone any notable change in the process of extraction, although its solubility in various media appears to be somewhat impaired.

The question whether the yellow colouring matter so obtained is a definite chemical entity is one to which it is very difficult to give a conclusive answer, chiefly on account of its physical properties. However, the uniform course of events observed on each of the many occasions on which the alcohol and ether process was carried out, strongly suggested that the product was a definite compound.

This view also received support from its behaviour towards its solvents and its precipitation by ether, as well as by its effect upon uric acid crystals, which is hardly what might be expected from a mixture of pigmentary substances.

The only fact with which I am acquainted which appears to be opposed to this idea is the impossibility of completely decolorising its solutions by certain metallic precipitants, which throw down the

* "The Influence of Colloids upon Crystalline Form and Cohesion," 1879, p. 52.

great bulk of the pigment; but, since we are ignorant of the form in which the pigment exists in such precipitates, *i.e.*, whether it is in actual chemical combination with the precipitant, this objection does not appear to be insuperable, especially as other pigments, which are certainly definite compounds, appear to behave in a similar way.

There can, I think, be little doubt that the same substance formed the basis of the products obtained by Thudichum, Tichborne, Schunck, and myself, such differences as were observed being due to varying degrees of purity, and probably to changes produced in the pigment by the various methods of extraction employed.

The most important respects in which my product differed from the urochrome of Thudichum were its behaviour with ether and when heated with mineral acids.

The fact that ether, when shaken with normal urine, does not acquire any yellow tint suggests, but does not prove, that, in its original condition, the yellow pigment is insoluble in ether, and at no stage of my process is the product soluble in that medium. Urochrome, on the other hand, is described by Thudichum as being more readily soluble in ether than in alcohol. Again, a portion of Schunck's product (urian) was also soluble in ether.

I have myself found that the pigment obtained from urine by saturation with baryta, precipitation with the acetates of lead, and extraction of the precipitate with cold dilute sulphuric acid, followed by immediate neutralisation with ammonia, is to some extent soluble both in ether and in chloroform, and can only attribute this difference to a change produced by the process of extraction employed.

When acted upon by hydrochloric or sulphuric acid upon a water bath, my product behaved more like those of Schunck than like urochrome. The chief difference from Thudichum's results was that no portion of the residue was soluble in ether with a red colour. I should, however, mention that I have repeatedly obtained a substance yielding a rich red ethereal solution, when the specimens treated had not been freed from indoxyl sulphate, but never when this impurity had been got rid of.

To the difficult questions connected with the origin and formation, in considerable quantities, of a pigment, such as is here described, the source whence it is derived, and the manner in which it enters the urine, of which it is a constant constituent, it is, at present, impossible to venture even a hypothetical answer; and we may well content ourselves for some time to come with the attempt to establish upon a firm basis the contention that the yellow colour of urine is not due to any of the band-yielding pigments, but to a distinct yellow colouring matter, of negative spectroscopic properties, which may be judged from its reactions to be a definite chemical entity. For the designa-

tion of this substance the name "Urochrome," assigned to it by Thudichum, appears eminently suitable.

The only points hitherto brought out which afford any clue to the chemical relationships of this pigment are the resemblance of the products of its decomposition to the humous substances described by Udránszky,* and the fact that it yields, when heated with nitric acid, a colour reaction which is indistinguishable from the xanthoproteic reaction, suggesting a relationship to the members of the aromatic series.

Udránszky classes Thudichum's uromelanine and the other products of the decomposition of urochrome as humous substances, and suggests as a possibility that the conversion of carbohydrates into such substances begins even within the body, and so may contribute to the yellow coloration of urine.

Certainly uromelanine has, as might be expected, certain obvious resemblances to the products which Udránszky obtained by the action of acids upon urine, and Thudichum long ago described how it might be prepared directly from urine by similar means. On the other hand, even if it be granted that the yellow pigment does yield humous substances on decomposition, any argument based upon this may well be regarded as open to the objection of explaining *ignotum per ignotius.*

IV. "Some Points in the Histology of the Nervous System of the Embryonic Lobster." By Edgar J. Allen, B.Sc. (London). Communicated by Professor W. F. R. Weldon, F.R.S. Received February 10, 1894.

The following observations have been made on late embryos of the common lobster (*Homarus vulgaris*) by means of Ehrlich's methylene blue method, as modified by Biedermann† and Apáthy.‡ The results to be recorded in the present communication apply chiefly to the thoracic ganglia, which in the embryo are fused into one mass.

The nerve elements, which have stained, may be divided into three main groups:—

I. Elements of which both the cell and the fibre lie entirely in the ganglionic chain, and which must be supposed to serve the purpose of co-ordinating the action of its various parts.

* 'Zeitschrift. f. Physiol. Chemie,' vol. 11, 1887, p. 537, and vol. 12, 1888, p. 33.

† Biedermann, "Ueber den Ursprung und die Endigungsweise der Nerven in den Ganglien wirbelloser Thiere," 'Jena. Zeitschr.,' vol. 25, 1891.

‡ Apáthy, "Erfahrung in der Behandlung des Nervensystems für histologische Zwecke," I, Methylenblau. 'Zeitschr. Wiss. Mikr.,' vol. 9, 189